Antibacterial effect of zinc oxide nanoparticles against water borne bacteria

Farwa Rubab, Muhammad Fayyaz Chaudhary and Noor Mohammad Butt
Preston Institute of Nano Science & Technology (PINSAT),
Preston University,
Sector H-8/4, Islamabad-44000, Pakistan
e-mail: nmbutt36@gmail.com

Abstract-During present studies we have investigated antibacterial properties of zinc oxide nanoparticles against gram negative bacteria isolated from various sources of drinking water. We have been able to isolate and identify five bacterial strains i.e. *Escherichia coli*, *Serratia marcescens*, *Enterobacter cloacae*, *Aeromonas hydrophila and Cronobacter sakazakii*. Our results showed that zincoxide nanoparticles of size 35nm have significant antibacterial effect. Cell culturability of all five bacteria was affected by ZnO nanoparticles at all concentrations (0, 100, 200, 300 and 500 µg/ml) tested. Most significantly growth of *E. coli* was inhibited and zone of inhibition was 28 mm at a concentration of 500 µg/ml. Zones of inhibition for *E.cloacae*, *S. marcescens*, *A. hydrophila and C. sakazakii*were24, 22, 19 & 20 mm respectively. When different volumes (0, 10, 20, 30, 40, 50, 60 µl) of the same concentration (500 µg/ml) of ZnO NPs were tested, highest zone of inhibition i.e. 25 mm was observed at 60 µl in *E. coli*. *E. cloacae*, *S. marcescens*, *A. hydophila* and *C. sakazakii* showed highest DZI of 19, 24, 15 and 18 mm at 60 µl. The bacterial strainswere also tested for identification of resistant isolates against different antibiotics. Among the antibiotics tested *E.cloacae* was found to be resistant against Erythromycin(ERYC) showing DZI of only 7mm whereas the same bacteria was sensitive to ZnO NPs (500 µg/ml) and showed a zone of 24 mm. Therefore ZnO NPs have the potential for their use as a very effective antibacterial agent for antibiotic resistant bacteria and eventually for water purification.

***Keywords*:** Zinc oxide nanoparticles, antibacterial effect, DZI, antibiotic resistant.

1. INTRODUCTION

One of the most pervasive problems, plaguing people all over the world, is insufficient access to clean water and sanitation. Most of the natural resources of drinking water are found to be contaminated with various toxic materials and pathogenic microorganisms (Baruah& Dutta, 2009). According to a World Health Organization (WHO) report, about 12 million people die every year because of water borne diseases (http://www.who.int/infectious-disease-). About 90% of all diseases occurring in developing countries are associated with the intake of impure water contributing to nearly 4 billion reported cases of diseases contracted from water in the world (Gopal *et al*., 2007).

Disinfection of drinking water is currently being carried out through physical and chemical techniques like chlorination, ozonation, UV treatment, filtration etc. However, these conventional methods are often chemically, energetically and operationally intensive. A good approach for developing countries is to encourage advance nanotechnology in water purification for safe drinking. Among the metal oxide nanoparticles, zinc oxide is interesting because of its vast applications in various areas. ZnO nanoparticles have been reported to have antibacterial effect (Xin *et al*., 2004; Vigneshwaran *et al*., 2006; Fei *et al*., 2006). ZnO nanoparticles have broad spectrum of antibacterial activity against both Gram-positive and Gram-negative bacteria, such as *Listeria monocytogenes*, *Salmonella*, *Escherichia coli*, and *Staphylococcus aureus* (Jones *et al*., 2008; Liu *et al*., 2009).

In vitro studies have shown that ZnO nanoparticles exhibit strong antibacterial activity against the pathogen indicator bacterial strain *E. coli* K88 (Wang *etal*., 2012). ZnO nanoparticles show exclusive toxicity to bacteria but exhibit minimal effects on human cells (Reddy *et al*., 2007). Zinc oxide is generally regarded as a safe reagent to humans and animals (Liu *et al*., 2009; Berube, 2008). This study, therefore, is aimed to evaluate the antibacterial effect of ZnO nanoparticles against human pathogens isolated from drinking water supply of Islamabad, the capital of Pakistan and to compare to antibiotics under laboratory conditions.

2. MATERIALS & METHOD

2.1 Purchasing of zinc oxide (ZnO) Nanoparticles

Zinc Oxide, dispersion Cat # 721077 nanoparticles of size<100 nm particle size (DLS), <35 nm avg. part. Size (APS) and 50 wt. % in H_2O were purchased from M/s Sigma Aldrich.

2.2 Purchasing of Antibiotics

Antibiotics used in this study include ciprofloxacin, ceftriaxone, erythromycin and trimethoprim-sulfamethoxazole which were purchased from M/s Oxoid and Liofilchem.

2.3 Isolation of bacterial strains

Bacterial strains used in this study were isolated from drinking water supplies of different sectors of Islamabad, Pakistan and identified by API 20E method. These identified strains were Gram negative including *Enterobacter cloacae*, *Escherichia coli*, *Serratia marcescens*, *Aeromonas hydrophila and C. sakazakii*.

2.4 Antibacterial Assay of ZnO NPs

Antibacterial assay of ZnO NPs was performed using agar well diffusion method (Perez *et al.,* 1990). Bacterial strains to be tested were streaked on nutrient agar plates.After that, 24 hours culture was used to form bacterial suspension. Turbidity of the suspension was compared with 0.5% Mcfarland solution. One ml of inoculums of bacterial strains was seeded on nutrient agar plates to get 10^6 CFU/ml. Lawn of bacterial strain was made on sterile nutrient agar plates using sterile cotton swabs. Wells of 11 mm were made using sterile borer on nutrient agar plate. Working volume 60 µl of different concentrations i.e. 0, 100, 200, 300 & 500 µg/ml of ZnO NPs sample was poured in separate wells. Also different working volume i.e.0, 10, 20, 30, 40, 50 & 60 µL of ZnO NPs of same concentration (500µg/ml) was poured in separate wells respectively. Diameter of zone of inhibition (DZI) was measured after 24 hours of incubation at 37ºC. Experiment was performed in triplicate to get more reliable results.

2.5 Antibiotic susceptibility Assay

Antibiotic susceptibility assay was performed by Kirby Bauer's disc diffusion method (Bauer *et al.,* 1966). Bacterial strains to be tested were streaked on nutrient agar plates. After that, 24 hours culture was used to form bacterial suspension. Turbidity of the suspension was compared with 0.5% Mcfarland solution. Lawn of bacterial strain was made on sterile nutrient agar plates using sterile cotton swabs. Pre-sterilized discs of 5 mm of antibiotics ciprofloxacin (CIP-5 µg), ceftriaxone (CRO-30 µg), trimethoprim-sulfamethoxazole (SXT- 25 µg) and erythromycin (15 µg) were placed on the surface of the bacterial lawn. The plates were incubated at 37 ºC for 24 hours. Diameter of zone of inhibition (DZI) was measured after 24 hours. Experiment was performed in triplicate to get more reliable results.

3. RESULTS

3.1 Growth and Morphological studies of isolated Bacterial strains

Bacterial strains isolated from drinking water supplies of different sectors of Islamabad, Pakistan were cultured on Nutrient Agar and Eosin Methylene Blue (EMB) agar. The plates were incubated for 24 hours at 37ºC. Growth pattern was observed after 24 hours. Round colourless colonies were observed on nutrient agar plates. While on EMB agar *E.coli* showed distinctive metallic green sheen color while other strains showed round colonies with varying shades of pink color. The growth of bacterial strains on EMB agar is shown in Fig.1.

a. Escherichiacoli ***b. Serratiamarcescens***

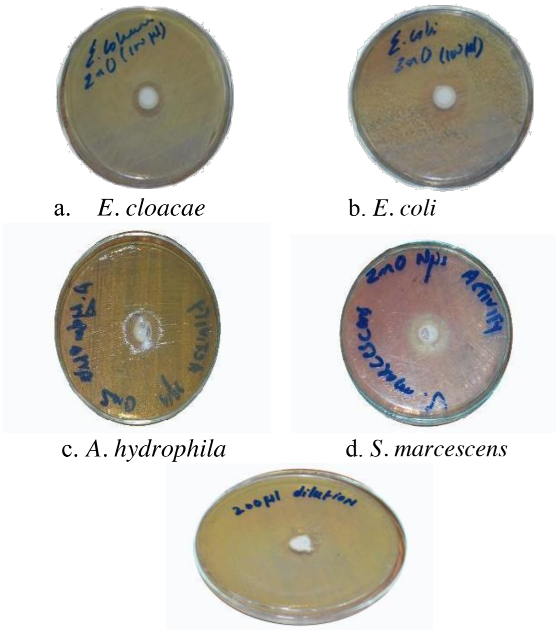
c. Enterobacter cloacae ***d. Aeromonashydrophila***

e. Cronobactersakazakii

Fig.1. Representation of Bacterial Growth of a. *Serratiamarcescens* b. *Escherichia coli* c. *Enterobacter cloacae* d. *Aeromonas hydrophilla* and *e. Cronobacter sakazakii* on Eosin methylene blue (EMB) agar

3.2 Antibacterial assay

Antibacterial activity of ZnO nanoparticles was tested against five identified strains i.e. *E. coli, S. marcescens, E. cloacae, A. hydrophila& C. sakazakii* isolated from drinking water supplies of Islamabad, Pakistan. ZnO nanoparticles showed marked antibacterial activity against all strains as shown in Fig.2 and Fig.3.



a. *E. cloacae* b. *E. coli*

c. *A. hydrophila* d. *S. marcescens*

e. C. sakazakii

Fig.2 Antibacterial activity of ZnO NPs (Conc. 500 µg/ml) on a. *E. colacae* b. *E. coli* c. *S. marcescens* d. *A. hydorphila* and *e. C. sakazakii*

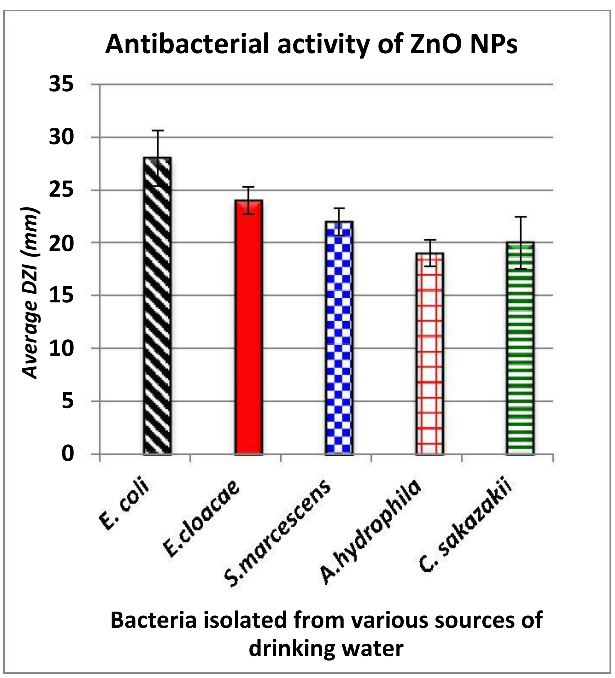


Fig.3 Antibacterial activity of ZnO NPs on water borne bacteria

When different working volumes i.e. 0, 10, 20, 30, 50, 60 µL of ZnO nanoparticles of concentration 500 µg/ml were poured in wells, *E. coli* showed highest zone of inhibition of 25 mm when 60 µl was poured in well.

E. cloacae, *S. marcescens, A. hydrophila* & *C. sakazakii* showed zones of inhibitions of 19, 24, 15 &18 mm respectively when 60 µl of ZnO NPs of concentration 500 µg/ml was poured in well as shown in Fig.4a. This shows that antibacterial effect of ZnO NPs is more significant with increase in volume poured in well. When different concentrations (0, 100, 200, 300 & 500 µg/ml) of ZnO nanoparticles were poured in wells highest zone of inhibition was observed against *E.coli* which showed diameter of zone of inhibition (DZI) of 28 mm at a concentration of 500 µg/ml. Antibacterial effect of ZnO NPs against the pathogenic strain of *Escherichia coli* O157:H7 has been reported in different studies (Xie et al., 2011; Jiang et al., 2009; Liu et al., 2009). While *E.cloacae*, *S.marcescens, A. hydrophila & C. sakazakii* showed diameter of zone of inhibition (DZI) of 24, 22, 19& 20 mm respectively at a concentration of 500µg/ml as shown in Fig.4b.

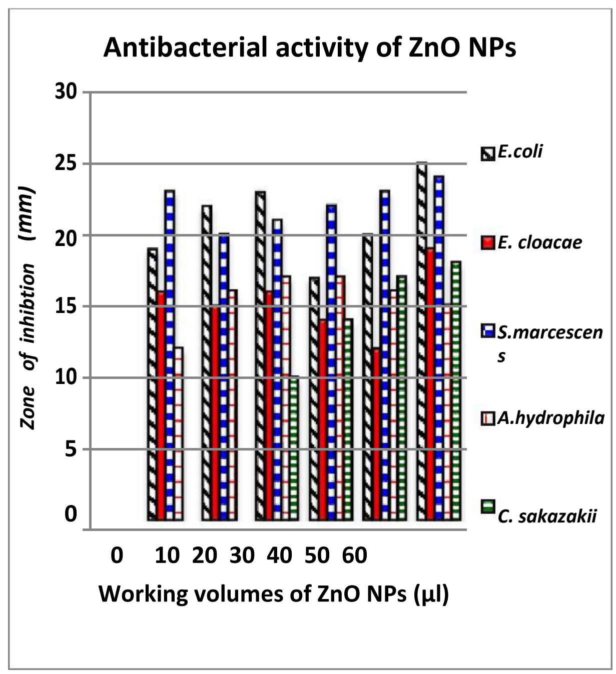


Fig.4a Antibacterial effect working volumes (0-60 µL) of ZnO NPs (Conc. 500 µg/ml) on different bacteria

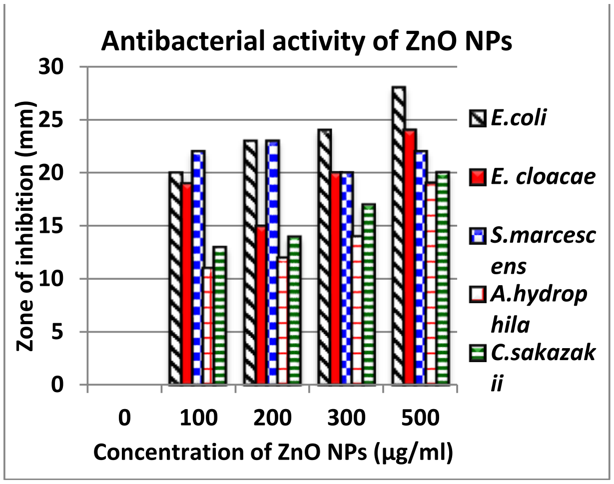


Fig.4b Antibacterial effect of different concentrations of Zinc Oxide nanoparticles on different bacteria

The bacterial isolates i.e. *E. coli, S. marcescens, E. cloacea, A. hydrophila & C. sakazakii* were also tested for susceptibility against antibiotics to compare the antibacterial activity of ZnO nanoparticles with antibiotics. All bacterial strains were sensitive to the antibiotics tested. The antibacterial effect of antibioitics; ceftriaxone (CRO), ciprofloxacin (CIP), trimethoprim- sulfamethoxazole (SXT) and erythromycin (ERYC) on these water borne bacteria is shown in Fig.5.

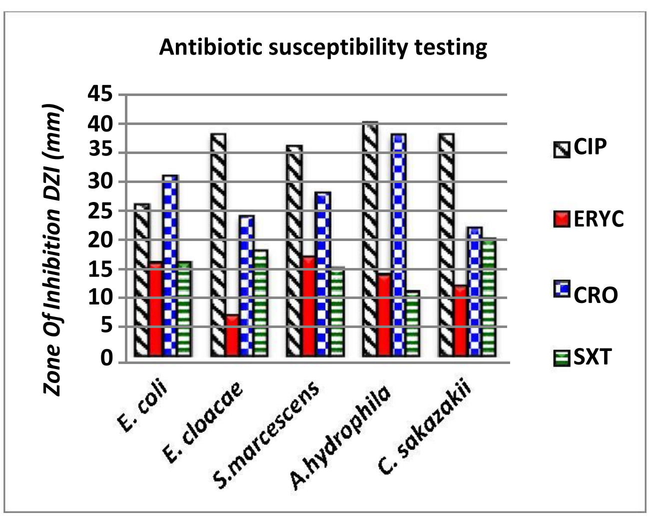


Fig.5 Antibacterial activity of different antibiotics on water borne bacteria

Erythromycin (ERYC) showed intermediate activity against all bacterial strains with the highest zone of inhibition of 17 mm against *S. marcescens* and minimal activity against *E. cloacae* which showed only 7 mm diameter of zone of inhibition. Trimethoprim- sulfamethoxazole (SXT) also showed intermediate antibacterial activity showing average zone inhibition 15 mm as compared to ciprofloxacin (CIP) which showed highest zone of inhibition 40 mm against *A. hydrophila* & ceftriaxone (CRO) which showed 38 mm DZI against *A. hydrophila* (Fig.5)

The comparison of the two antimicrobial agents i.e. zinc oxide nanoparticles and antibiotics against waterborne bacteria revealed that bacteria which showed resistance against antibiotics were susceptible to nanoparticles, as in case of *E. cloacae*. This bacterium was found to be resistant against antibiotic Erythromycin (ERYC) showing 7 mm DZI, whereas it was sensitive and showed a 24 mm zone of inhibition against ZnO nanoparticles (Fig.6.). This highlights the fact that nanoparticles of zinc oxide are effective in the inhibition of bacteria which have developed resistance against antibiotics.

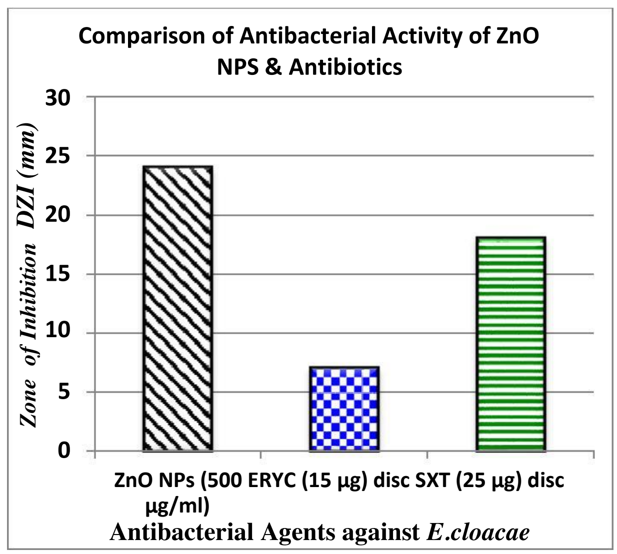


Fig.6 Comparison of antibacterial effect of ZnO NPs & antibiotics

Conclusion

Zinc oxide nanoparticles are effective against all the tested strains of bacteria isolated from drinking water supply of Islamabad. *E. coli* and *E. cloacae* were most effectively inhibited by ZnO NPs showing a 24 & 28 mm diameter of zone of inhibition. These results showed that antibacterial effect of ZnO NPs is more significant with the increase in volume poured in well.

Among different concentrations tested highest zone of inhibition was observed at 500µg/ml which indicates that antibacterial effect increases with increase in concentration. Among the different antibiotics tested ceftriaxone (CRO) and ciprofloxacin (CIP) were effective against all the bacterial isolates, whereas trimethoprim- sulfamethoxazole (SXT) and erythromycin (ERYC) showed intermediate results against all bacterial strains except *E. cloacae* in which erythromycin (ERYC) showed only 7 mm zone of inhibition. These results will be helpful in understanding the usefulness of nanoparticles against problematic and antibiotic resistant bacteria in future.

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